

# NEAR-INFRARED SPECTROSCOPY OF INDIVIDUAL COW MILK AS A MEANS FOR AUTOMATED MONITORING OF UDDER HEALTH AND MILK QUALITY

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## ABSTRACT

An urgent need exists to develop simple analytical on-line system which will identify milk composition and mastitis on the farm, to help farmers when take managerial decision. So far, Mid-infrared and Near-infrared(NIR)(1100nm to 2500nm) spectral analysis have been explored to measure milk constituents in whole milk samples. Electroconductivity has been used for on-line mastitis detection. Results of this study indicate the feasibility of the near NIR range(680nm to 1235nm)for both: mastitis diagnosis and cow milk composition analysis. Transmittance spectra of 200 unhomogenized bucket milk samples of 20 cows and 160 quarter foremilk samples from five mastitis cows were examined for five months and four consecutive days, respectively. NIR mastitis diagnosis was carried out at a very early stage with high repeatability. The accuracy was 95% when compared with somatic cell count(SCC).A high correlation coefficient was obtained for each constituent of milk (fat, protein, SNF, lactose, SCC) for every cow, between near NIR and laboratory measurement ( $R>0.9$ ).

KEYWORDS: Near- infrared transmission, cows milk, mastitis, constituents analysis.

## INTRODUCTION

For the purpose of the man's safety improvement, the dairy industry needs to emphasize to high quality products. Most milk quality problems originate on the farm and usually can not be erased by further processing. New approaches in milk quality testing are still needed on the farm to reveal the deteriorated milk and cows health disorders. Management of dairy cattle-breeding involves rapidly automated systems for the control of the entire breeding process(Rossing, W. & B. Ipema, 1989). On-line control of milk is already used to determine temperature and electrical conductivity for mastitis detection and also milk quantity (Rossing, W. et al., 1989; Rossing, W. & B. Ipema, 1989; Maaije, K. et al., 1992). Near-infrared spectroscopy is a non-destructive method, which has recently developed as a rapid and simple method for determining the compositions of various types of food and feed (Williams, P.C., 1990). This method has been applied to measure the contents of various constituents in dairy products(De Boever, J.L. et al., 1990; Sato, T. et al., 1987; Williams, P.C., 1990; Schmilovitch, Z. et al., 1992).

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In previous papers(Kyrlin,N.I. & R.N.Tsenkova,1980; Tsenkova,R., 1988; Tsenkova,R. et al.,1988; Tsenkova, R.N. et al.,1992) the near NIR range was examined in comparison with conventional methods for mastitis diagnosis and milk component analysis. It was found to have high reliability for mastitis diagnosis, using unhomogenized milk samples.

This paper reports on research to examine the feasibility of the near NIR range for simultaneous milk composition analysis and mastitis diagnosis at very early stage with high repeatability, using individual cows milk.

## MATERIAL AND METHODS

### Milk Samples and Spectra

In this study quarter and bucket ( taken from the milk-yield meter) individual milk samples of 20 Holstein cows were analyzed for 5 months, from September 1991 to February 1992. From these cows a total of 360 unhomogenized milk samples, obtained at Obihiro University Farm was analyzed by Pacific Scientific Spectrophotometer - NIR System, model 6250. Prior to analysis each sample was warmed to 40 °C in a water bath. The NIR transmittance spectra (T) were collected in terms of optical density -OD, i.e.  $OD=\log(1/T)$  with a wavelength range of 680nm to 1235 nm and 700 data points per sample. Absorbance data -  $\log(1/T)$  were stored in the linked computer. For NIR data, a quartz cuvette with walls 1mm thick and containing a milk sample of 4mm thick was used. Spectra were obtained as the average of 50 scans. The other duplicate samples were sent to the Milk Testing Laboratory in Obihiro (Japan) for milk constituents analysis. Each sample (50ml) was thoroughly mixed before division into subsamples for the various analysis. Two experiments were carried out.

### Experiment 1

A total of 200 individual morning and afternoon milk samples from the entire udder were taken from 20 cows that were monitored continuously for 5 months. These samples were obtained monthly, from each cow milk-yield meter after milking was finished.

### Experiment 2

A total of 160 quarter foremilk samples were collected from each test of 5 mastitis cows. This entailed sampling each cow, before the morning and evening milking, for four consecutive days. Five cows with the highest level of SCC after the regular monthly checking were selected for this particular experiment.

### Chemical analysis

The duplicate milk samples sent to the Milk Testing Laboratory were analyzed for milk constituents by Milko-scanner and Foss-somatic ( N.Foss- Electric A/S Hillerød DK3400 - Denmark). The samples were analyzed for: fat(F), protein(P), lactose(L), solids non fat(SNF) and somatic cell count (SCC). Log(SCC) was used for calibration work.

### Data treatment

The scattering induced by the particles in milk showed a distinct individual pattern for each cow. On-line measurement of milk components does not allow for the analysis of homogenized milk. To avoid the influence of these factors in our two experiments, we analyzed the data of spectra of each cow separately. The data of each cow was stored in its respective file. The file names correspond to the cow number. For comparison, a mixed data file containing spectral data from

different cows was treated. In the first experiment 20 files from 20 cows and one mixed file composed of individual milk spectral data of all 20 observed cows, taken after morning milking in September were analyzed. The file was referred to as: IMIX-(individual mixed). The number of spectra in the files which were compared and analyzed was the same.

Preliminary calculations showed that a better sample component analysis could be obtained by using the second derivative of the  $\log(1/T)$  absorbance curve rather than  $\log(1/T)$ . All data were therefore analyzed quantitatively by using the second derivative transformation. To evaluate the feasibility of the use of NIR spectroscopy for quantitative analysis of the main milk components, multiple linear regression analysis was carried out on each of the sets of reference data on the duplicate readings of the five means ( four from Milko-scan and one from Foss-somatic). The criteria used for evaluation was a high multiple correlation coefficient (R) and a low standard error (SE). For qualitative analysis, to detect mastitis, foremilk spectra of the udder quarters of the examined cows were compared. To highlight the spectra differences when one or more quarters of the same udder were mastitic, the Spectral Function (F) was created using mathematical treatment of raw absorbance spectral data at four wavelengths in the near NIR range. The examined milk samples were defined as a mastitic samples when their  $SCC > 500$  000/ml.

## RESULTS AND DISCUSSION

Original spectra of individual, bucket milk samples of mastitis cow No.664 and healthy cow No.698 (Fig.1; Table 1) and cow No.686(Fig.2,3) showed that the spectra from the same cow are similar. However, the groups of spectra of different cows are different.

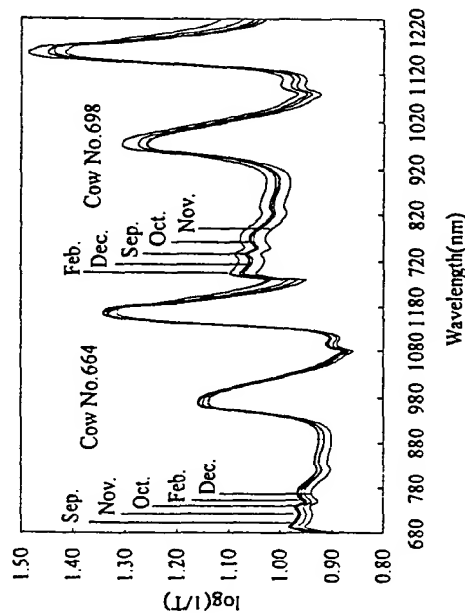


Fig. 1. Raw spectra of bucket milk samples of mastitic cow No.664 and healthy cow No.698 .

Table 1. SCCx1000/ml of bucket milk samples of mastitic cow No.664 and healthy cow No.698

Cow No.	Somatic cell count per month (1000/ml)				
	Sep.	Oct.	Nov.	Dec.	Feb.
664	913	171	408	574	1225
698	9	6	21	10	16

Differences in the shape of the curves are most apparent between 750 to 970nm (lactose, protein and water absorption band), also, between 1018nm to 1188nm (protein and fat absorption band), (Williams, P.C. & K. Norris, 1989). Mastitis causes an unbalance in milk composition, i.e. changes in all milk components (Barbano, D., 1989). These changes occur as corresponding multiplicities in the whole NIR milk spectra.

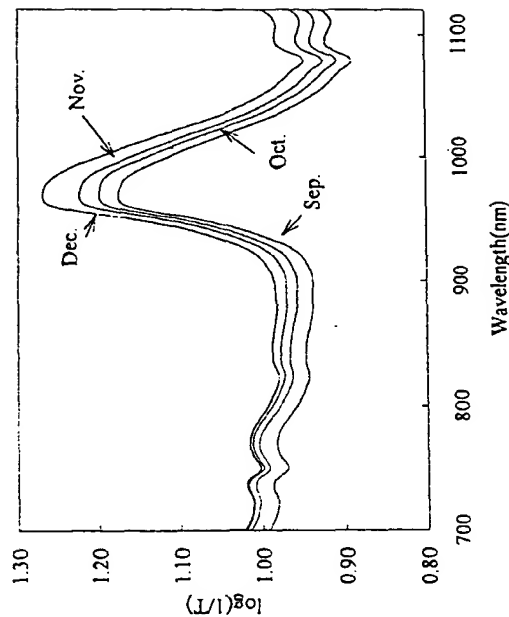


Fig.2. Raw spectra of bucket milk samples of cow No.686 for four consecutive months: September, October, November, December.

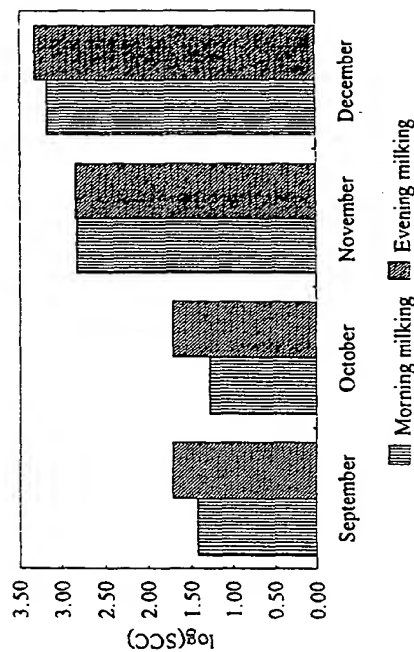


Fig.3 Log SCC of morning and evening bucket milk samples of cow No.686 for four consecutive months.

Original spectra of bucket milk samples of cow No.686, (Fig.2,3;Tabl.3), showed similar spectral curves when the examined cow did not have mastitis( SCC was low in September and October) and - different spectral patterns, when the disease occurred (in November and December).

Table 2. SCC in 1000/ml of morning and evening bucket milk samples of cow No.686 for four consecutive months

Sampling time	Somatic cell count (1000/ml)			
	Sep.	Oct.	Nov.	Dec.
Morning	27	19	678	1530
Evening	52	52	709	2098

When all udder quarters were healthy, their foremilk spectral curves were also similar,(Fig.3,4).

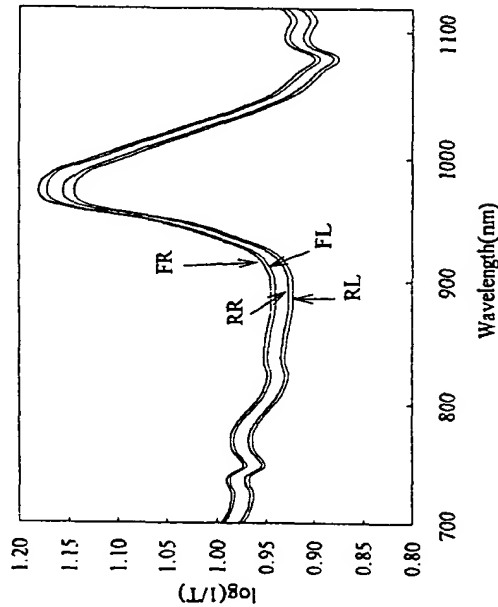


Fig.4 Raw spectra of the udder quarters foremilk samples of healthy cow No.686, in October, when SCC is still low(SCC<500 000/ml); FL-front left; RL-rear left; FR-front right; RR-rear right.

When SCC of bucket milk samples and foremilk samples of rear right and rear left udder quarters of cow No.686 increased (in November and December), different patterns of foremilk spectral curves by healthy and mastitis quarters, respectively, were observed (Tabl.2,3;Fig.5,6; ) in the same range of wavelengths as was found for individual bucket milk of other cows,(Fig.1).

Table 3. SCC in 1000/ml of quarter foremilk samples of mastitic cow No.686 for four consecutive days

Cow No.686	Somatic cell count (1000/ml)			
Quarters	18 Dec.	19 Dec.	20 Dec.	21 Dec.
Front Left	26	11	24	32
Rear Left	78	15	58	74
Front Right	26	10	12	23
Rear Right	9009	15126	10174	3791

It was found that the foremilk spectra of the respective quarter changed as soon as its SCC had been increased when compared with the other quarters with stable and low SCC(Fig.5).As an example, the Spectral Function F of the front right(FR) quarter changed at very early stage, when the SCC increased, but was still lower than 500 000/ml,(Fig.7).

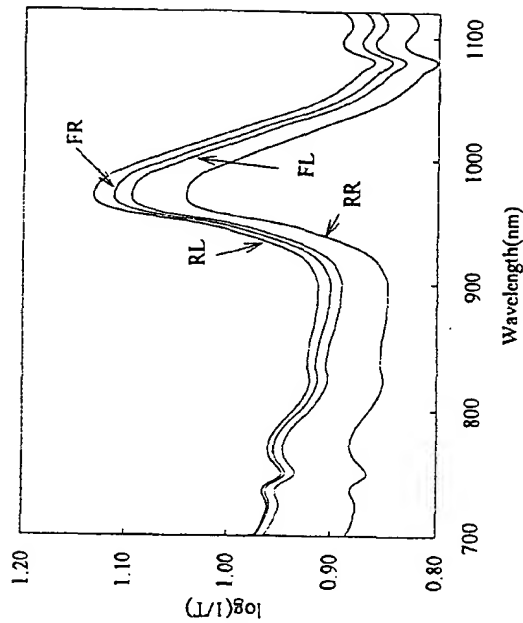


Fig.5 Raw spectra of the udder quarters foremilk samples of mastitic cow No.686 in November (SCC>500 000/ml).

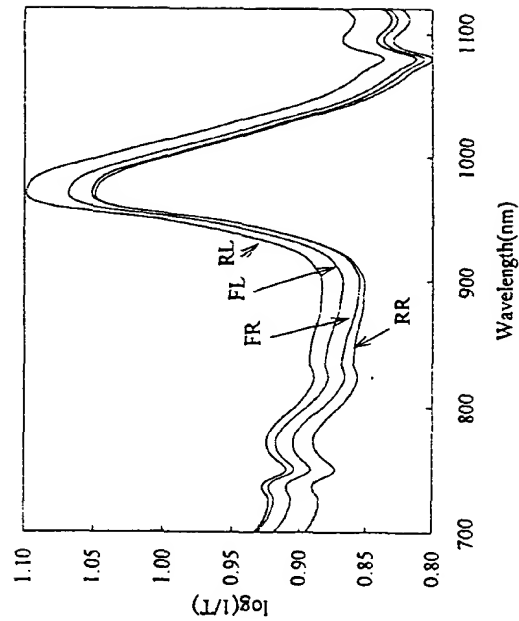


Fig.6 Raw spectra of the udder quarters foremilk samples of mastitic cow No.686 in December(SCC>500 000/ml).

The accuracy of NIR mastitis diagnosis was 95%, when the Spectral Function F was used as a criteria and compared with SCC. NIR method had similar response like SCC and the same repeatability (Fig.8,9),but essentially high sensitivity (Fig.7).

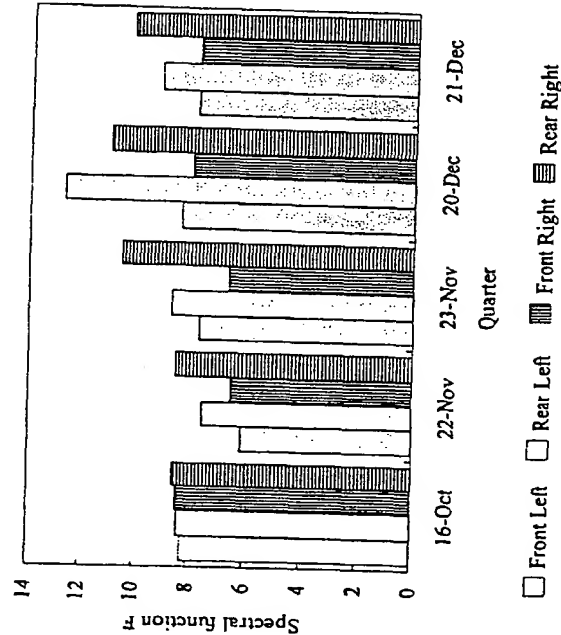


Fig.7 Spectral Function F of cow No.686 foremilk samples corresponding to different physiological conditions of the udder quarters.

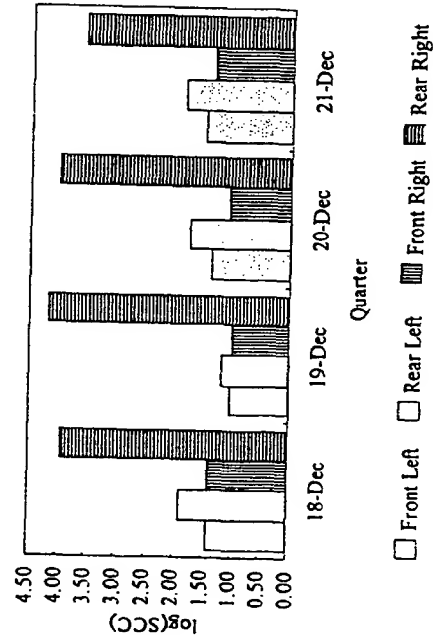


Fig.8 Log SCC of the udder quarters foremilk samples of mastitic cow No.686 for four consecutive days in December.

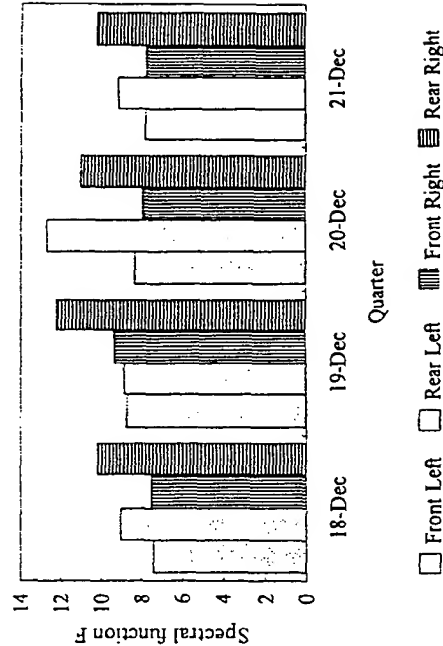


Fig.9 Spectral Function F of cow No.686 corresponding to different SCC level of the udder quarters milk samples for four consecutive days in December.

The results of the regression analysis, obtained for respective constituents when 20 cows were used, showed high correlation for individual cow data, even though the spectra for these files were taken once a month after morning and evening milking for five consecutive months. Table 4 represents a sample of data obtained from five of the 20 examined cows and data from the mixed file(IMIX), which had a notably lower correlation coefficient and higher standard error, compared with other groups. Variation between spectra of individual cow milk samples seems to depend on individual variation rather than on composition.

Table 4. Individual milk - correlation coefficient(R) and standard error.

Data file	Constituents:									
	Fat		Protein		Lactose		SNF		log(SCC)	
	R	SE	R	SE	R	SE	R	SE	R	SE
I498	0.99	0.05	0.98	0.05	0.96	0.04	0.99	0.02	0.98	0.29
I538	0.99	0.08	0.99	0.02	0.97	0.07	0.95	0.08	0.99	0.03
I567	0.99	0.24	0.99	0.03	0.97	0.08	0.99	0.03	0.98	0.18
I610	0.99	0.19	0.99	0.03	0.95	0.07	0.96	0.09	0.98	0.24
I631	0.99	0.16	0.99	0.03	0.99	0.02	0.98	0.05	0.88	0.05
IMIX	0.86	0.55	0.78	0.15	0.73	0.12	0.77	0.15	0.59	0.47

## CONCLUSIONS

The near NIR spectroscopy is better applied for milk composition analysis when spectra of individual milk samples of each cow are used separately. For further investigation, a calibration for each cow and prediction evaluation could be done with different set of samples by the same cow. A comparison of the NIR method and the standard method (counting somatic cells) for mastitis diagnosis revealed that these methods gave similar results. The advantages of NIR method over other mastitis test methods are its sensitivity, repeatability and simplicity. A threshold of the Spectral Function F, which will correspond to already existing standard of somatic cell count (SCC)-500 000/ml for mastitis diagnosis has to be determined in a future study. NIR method allows milk composition measurement and mastitis diagnosis to be done at the same time, using on-line optical sensors for milk control. On the basis of near NIR range (680nm to 1235nm), which extends the range of cheap optical sensors and fiber optics, different prototypes for milk quality control could be developed, depending on the purpose and the place of intended use. Having NIR on-line information for milk and feed quality and composition (Williams, P.C. & K. Norris, 1989; Williams, P.C., 1990), it could be possible to organize feedback and to adjust cow treatment and herd management accordingly. In other words, the use of NIR spectroscopy could be a further step towards full dairy automation.

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